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# SOME PARASITES OF *SIMULIUM* LARVÆ AND THEIR EFFECTS ON THE DEVELOPMENT OF THE HOST.<sup>1</sup>

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During the spring of the present year (1911) while studying entomology at the Bussey Institution of Harvard University, I made numerous collections of *Simulium* larvæ, which are extremely abundant in the neighboring streams, with the intention of studying the development of the imaginal discs which are unusually well defined in this genus of diptera. As, however, I found that a large percentage of these interesting larvæ were heavily parasitized by two very different organisms, namely a worm and a protozoön, I turned my attention rather more directly to these and their effects on their hosts, during the few weeks intervening between my first discovery of the parasites and the pupation of the insects.

Before giving any details I wish to take this opportunity to offer my sincere thanks to Professor Wheeler, who by his kind suggestions and advise enabled me to bring together the following facts, which, though very incomplete in form, do not appear to have been recorded before. My thanks are also due to Professor Johannsen for naming the species of *Simulium* larvæ and to Professor T. H. Montgomery who identified the worm parasite as a species of *Mermis*.

## LIFE HISTORY AND STRUCTURE OF *SIMULIUM* LARVÆ.

A brief summary of the structure, and mode of life, of the *Simulium* larvæ may not be out of place here.

If during the months of March to May one examines the rocks or vegetation in any swiftly flowing stream in the neighborhood of Forest Hills, Mass., especially where its bed causes a small cascade, one will, in all probability, observe a large, dark, gelatinous mass where the current is swiftest and the water

<sup>1</sup> Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 45.)

shallowest. On closer examination the mass will be seen to consist of numerous curiously shaped larvæ standing upright on the rock, to which the hind end of the abdomen is firmly attached. These larvæ, the largest known North American species of which measure when mature some 12 mm., have the following characters: The soft-skinned body is more or less cylindrical, though the posterior third is somewhat swollen. The segments are poorly defined but the first three behind the head, namely, the thoracic segments, are usually distinctly marked off from the following abdominal segments.

The prothoracic segment bears a single leg (Plate I., Fig. 1) which is apparently two-jointed; the distal joint is small and retractile and terminates in a sucker which is armed with numerous hooks. No other segment bears any trace of legs, with the exception of the apical abdominal segment where the pair of anal prolegs of some other larvæ is represented by a powerful, armed disk-like sucker, by means of which the larva firmly attaches itself to rocks or vegetation when at rest. The skin is usually of a greenish gray color in immature larvæ, but gives place to a reddish brown as they mature.

The head is sub-cylindrical and is usually of a darker color than the rest of the body. Its chitinous integument is much denser and less elastic than that covering the remainder of the body. This results, during growth, in an ecdysis of the head capsule alone, being necessary more frequently than that of the general cuticular covering of the body. The new head capsule exposed after such an ecdysis is perfectly pigmentless in *Simulium hirtipes*, though dark spots soon form on its vertex (Plate I., Fig. 2, *a*, *b* and *c*), followed by a gradual infuscation of the whole surface. Individuals were observed in which the almost black head capsule was half removed, exposing the new pigmentless capsule, entirely independently of the body cuticle, the ecdysis of which was never observed, except at pupation.

On either side of the head are two black eye spots and on the anterior border are two lateral fan-like organs borne on elongated pedicels, which are used by the larva in procuring food. These fans consist of numerous curved rakes (Plate I., Fig. 3) bearing on one side long stiff cilia which, when the fan is expanded,

stretch from one rake to the next, thus forming a very fine strainer which allows water to pass through it, but retains any small organisms, such as the diatoms, on which the larva subsists. By means of a curious flicking motion these fans can be closed and their contents brushed into the mouth orifice. Here are situated two large glands (Plate IV., Figs. 9 and 10 (*d*)) on the dorsal side of the pharynx, whose function appears never to have been determined. They are covered with an apparently porous membrane which is clothed with short stout hairs. It would seem that they secrete some sticky material onto these hairs which removes the particles of food from the rakes when the latter are brought in contact with them.

It is usually stated that the fans are used to set up currents in the water and thus sweep food toward the mouth. My observations, however, lead me to believe that they act as a "strainer" and this is supported by the fact that, living as these larvæ do, in the swiftest currents, such movements would be useless.

The salivary glands are very large and secrete powerful silken threads which are used by the larvæ as anchor lines to hold them in an upright position no matter how strongly the current of water may flow.

Although the larvæ appear to be very sluggish, they can move about actively on the rocks by a looping motion similar to that of the geometrid larva. It is interesting to note the care with which these larvæ, when thus moving about, assure themselves that the adhesive disc of the prothoracic leg is firmly attached before relaxing the anal disc and *vice versa*. Respiration is accomplished by means of retractile finger-like blood gills, normally three in number, which are situated on the dorsal surface of the last abdominal segment just above the anus. These gills, which can be distended at will by the insect, by means of blood pressure, are apparently inadequate for use in any but rapidly flowing water which contains plenty of oxygen, for if the larvæ be placed in a jar of still water very few will survive for more than eight hours, though life may be prolonged for two or three days by placing them in small numbers in Petri dishes containing only sufficient water to just cover them.

The form and size of the imaginal discs, or histoblasts, will here be described in detail as they are of especial interest in connection with the parasites.

The thoracic histoblasts are twelve in number and are very large and conspicuous in normal larvæ as they approach full growth (Plate I., Fig. 4).

The six discs to be found on each side of the thorax are the following:

1. Adult prothoracic leg histoblast—situated at the base of the larval prothoracic leg.

2. Adult mesothoracic leg histoblast—situated ventro-laterally on the mesothoracic segment.

3. Adult metathoracic leg histoblast—situated ventro-laterally on the metathoracic segment.

4. Adult wing histoblast—situated dorso-laterally on the mesothoracic segment. This in the mature larva is by far the largest disc and it soon comes in contact with the mesothoracic leg disc.

5. Adult halteric histoblast. This in the early stages is almost as large as the wing disc, but its growth and development are very slow and it soon disappears under the rapidly expanding wing disc.

6. Pupal respiratory tuft histoblast. This is situated on the prothorax and in its young stages has the appearance of being quite homologous with a prothoracic wing. It, however, soon begins to take on a definite form, and the comparatively stout tracheal tubes can be seen growing and lengthening beneath the transparent cuticle till they become coiled up as indicated in Plate I., Fig. 4.

It should be noted that this histoblast is not in the true sense of the word an "imaginal" disc since the respiratory filaments are exclusively pupal organs, and have no equivalent structure in any adult. Their similarity to a prothoracic wing destitute of the wing membrane is of interest. A few days before pupation this "pupal" histoblast begins to darken. Pigmentation commences at the apex of the folded tubes and slowly works backwards toward the base when the disc takes on the appearance shown in Plate III., Fig. 8*b*. Later, immediately prior to pu-

pation, the cuticle over this disc ruptures and liberates the fully formed and now functioning filaments.

The growth of these various histoblasts causes the thorax to swell considerably, thus giving the body the appearance of being constricted in the middle.

Internally there is comparatively little tissue. The abdomen contains the alimentary tract, and the much elongated salivary glands which lie normally in a ventro-lateral position with regard to the alimentary tract. The sexual organs are small and not easily found even in serial sections. The remaining portions of the body cavity are filled with blood plasma in which is suspended a quantity of fat body, mainly collected near the apex of the abdomen and causing this region to become slightly swollen. As the larvæ mature this fatty tissue very materially increases, and when dissected out, is of a stringy nature.

#### METHODS.

Most of the larvæ were killed as soon as they were brought into the laboratory, but a few of the more heavily parasitized ones were kept alive in running water by covering the mouth of a jar with fine netting and introducing a piece of rubber pipe into the jar, through which tap water was run. In this manner specimens were kept alive for several days.

The following killing fluids were found to be the most satisfactory among several tried:

1. *Hot Water*.—Water was just brought to the boil when the larvæ were immersed and allowed to cool in the water. This method was most unsatisfactory from a histological point of view, but it had the advantage of leaving the skin as transparent as in its normal condition. It was also possible to dissect larvæ thus killed.

2. *Gilson's Fluid*.—This was used hot as described above and gave good results, but had the disadvantage of making staining with the hæmatoxylin difficult.

3. *Kahle's Fluid*, consisting of 30 parts water, 15 parts 96 per cent. alcohol, 6 parts formalin (40 per cent.), 1 part glacial acetic acid. This fluid has been recommended by W. Kahle (1908) and proved to be superior to Gilson's fluid both for

fixing and staining, and in the end was exclusively used. It was also used hot.

The chief advantage of both of these fluids was that, besides giving excellent histological preparation, they caused the histoblasts to turn milky white so that their position and form could be readily observed immediately after the specimens were killed.

*Staining.*—Heidenhain's iron hæmatoxylin combined with orange G was almost entirely used as it gave the best differentiation, though staining was rather slow. Replacing orange G with eosin accelerated staining but differentiation was less precise.

#### MERMIS SP. PARASITIZING SIMULIUM LARVÆ.

A number of larvæ were placed in a jar of water and left over night. The following morning I was surprised to see several white worms moving about at the bottom of the jar. It was evident that these had come out of the *Simulium* larvæ, so I went out to a ripple where the larvæ were particularly abundant and examined the colonies on several stones. I then noticed the large size of many individuals, and on examining these I found that many of them had a worm coiled up within the abdomen (Plate I., Fig. 5). When a quantity of material was brought into the laboratory it was found that these parasitized larvæ were much more sluggish than their healthy companions, which rapidly explored the jar in which they were confined, with their peculiar looping gait. The parasitized individuals, however, seemed much less concerned as to their new surroundings, and many of them were soon motionless, standing upright on the bottom and sides of the jar with their rakes expanded, ready to catch any food which might float their way. Here is a possible explanation of their larger size. Owing to their curious form of feeding it is evident that the more sluggish an individual is the more food it can obtain, and should it grow but a little larger than its companions, it will reach above their innumerable outstretched rakes, so that its food supply will be very materially increased.

It must not however be taken for granted that all parasitized larvæ were larger than their healthy companions, for some remained quite small, whereas many of the larger larvæ showed no

signs of worm parasites. As a general rule, however, the largest larvæ were found to contain one or more worms coiled up within the abdomen. One would naturally expect this rule not to be very constant, if, as conjectured, it is simply due to a more sluggish temperament and increased appetite on the part of the parasitized larvæ.

A case of a *Mermis* parasitizing ants was described by Professor Wheeler ('07) and here also he noticed a great increase in size of the host due to a greatly increased appetite during the larval stage.

A number of the worms were dissected out and sent to Professor T. H. Montgomery who pronounced them to belong to an undetermined species of *Mermis*.

#### RETARDATION OF DEVELOPMENT OF THE HISTOBLASTS DUE TO MERMIS.

On making a closer examination of the parasitized larvæ a far more remarkable effect than that of increased size was noticed, for it was found that the presence of *Mermis* parasites, no matter in what numbers, has a direct effect on the development of the histoblasts. In a normal larva of about 10-10.5 mm. which is the maximum length, and is attained immediately prior to the blackening of the respiratory filament histoblasts, the latter are quite large and owing to their white color readily visible to the naked eye; especially when the larva has been killed in Kahle's or Gilson's fluid (Plate II., Fig. 6). If a parasitized larva of the same, or greater, size be examined no trace of the histoblasts can be discovered with the naked eye, and can only be detected with difficulty under a dissecting lens. Under the low power of a compound microscope, however, they are seen to be represented by small white traces of the organs which should at this time be far advanced in development (Plate II., Fig. 7). A close examination fails to reveal any differentiation of these retarded histoblasts into the component parts of the adult, or pupal, organ.

The parasitized larva rarely develops beyond this stage, though I have observed specimens which were turning reddish brown, and contracting slightly as a healthy larva would, shortly before

maturation. In some cases, even, the rudimentary respiratory filament histoblast begins to darken in color.

I examined numerous specimens from the same stream at Forest Hills, and, with slight variations in intensity, they all showed this nearly complete suppression of the imaginal discs. Some weeks later I chanced to pass a small stream at Norwood, about seven miles from Forest Hills, and seeing *Simulium* larvæ present in small numbers, I took nine specimens from the rocks in order to see whether the species was identical with that common in our streams at Forest Hills. They proved to belong to the same species, namely, *Simulium hirtipes*, but I was much surprised to find that here also the *Mermis* parasite was much in evidence for of the nine specimens taken, six contained the worm. The effects however in these specimens were not so marked as in those found nearer home, for four of the six had turned brown and although the histoblasts were much smaller than is normally the case they were readily visible to the naked eye. Undoubtedly, however, maturation was impossible, for even should the larva manage to pupate it would soon die for want of oxygen, since the respiratory filaments were but half formed.

In a third locality, the Stonybrook Reservation near Forest Hills, a different species of larva was found. Professor Johannsen states that this larva is quite new to him and may be the undescribed larval stage of *S. bracteatum*, which occurs frequently in this State, but as I found only adults of *hirtipes* it was impossible to confirm this supposition. These larvæ were, however, also found to suffer from *Mermis* parasitism, but to a much less extent than those of *S. hirtipes*. The effects on the host were, however, as one would expect, identical with those on *hirtipes*, though the increased size was not so evident.

A large number of larvæ were measured, cut open and the worms removed. It is interesting here to note that even in fixed specimens where the fluid had caused the skin to become opaque, the presence or absence of worms could in all cases be determined with certainty by a glance at the histoblasts. The worm, it should be noted, did not cause the abdomen to swell up or become distorted to any appreciable degree.

A few selected results shown by the examination are appended:

	Length.	Color of Larva.	Histoblasts.	No of Worms.	Size of Worms.
1	10.75 mm.	Gray.	Minute.	12	Av. .75-1 cm.
2	11.50 mm.	Gray.	Minute.	1	2.75 cm.
3	11.0 mm.	Gray.	Minute.	1	3.00 cm.
4	11.0 mm.	Gray.	Minute.	3	1.3, 1.5, 1.5 cm.
5	10.5 mm.	Brownish.	Minute.	1	3.00 cm.
6	9.0 mm.	Gray.	Quite small.	3	.9, 1.3, 1.5 cm.
7	8.5 mm.	Brownish.	Large.	0	—
8	10.5 mm.	Gray.	Large.	0	—
9	8.0 mm.	Brownish.	Large.	0	—
10	7.0 mm.	Brownish.	Large.	0	—
11	8.5 mm.	Gray.	Large.	0	—
12	11.0 mm.	Gray.	Large.	0	—

From this table it will be seen that when there is but one worm in a host it attains a length of about 3 cm. during its parasitic life; that is, roughly about three times the length of the host itself, since the average length of a parasitized larva is 10.5-11 mm. It will also be seen that in one case as many as twelve worms were removed from a single host, and that in this case they all remained small, owing doubtless to insufficient food supply. In other instances, however, where several worms were present in one host it was noticed that one had attained almost to the normal size of 3 cm. whereas the remaining worm or worms were less than 1 cm. in length.

The larval measurements showed that parasitized larvæ average about 11 mm. (Plate III., Fig. 8a) whereas mature, and therefore somewhat contracted, larvæ, average some 8-8.5 mm. (Plate III., Fig. 8b), though a few are abnormally large.

#### LIFE HISTORY OF THE WORM.

As yet nothing is known of this except during the latter part of the life in its host. In all probability the eggs or young worms, are caught as they float down stream by the outspread larval rakes and are swept into the mouth, passing into the alimentary tract through the walls of which they bore their way till they enter the body cavity. It should however be noted that no scars or hypertrophy of the wall of the alimentary tract were noticed in several serial sections made of parasitized larvæ. The alternative hypothesis is that the larval worms bore into their hosts through the thin cuticular covering of the ab-

domen. That they should be present in the *Simulium* eggs is hardly possible, and this hypothesis can be with safety rejected.

Either of the former hypotheses offers a possible explanation for the variation in development of the histoblasts of parasitized larvæ taken from different streams, for in the case of those taken at Norwood where the discs were of a moderate size, the worms may not have entered the body cavity till the discs were somewhat developed. Then again where one worm has grown to nearly the normal size of 3 cm. whereas other worms in the same host have remained small, it is probable that the large worm entered the body cavity some time before the others.

Figure 9, Plate IV., which is a drawing of a median longitudinal section of a parasitized larva, shows the ventral position of the worm with regard to the alimentary tract, though it will be seen that the mesenteron has been somewhat pushed to one side by the coiled parasite. It will also be seen by comparing Fig. 9, Plate IV., with Fig. 11, Plate III., that the parasitized larva has much less fat body than that of a healthy individual at about the same stage of growth.

Another effect of the host is that apparently the sexual organs do not develop in parasitized larvæ. These are not easily seen in a healthy *Simulium* larva, as they are very small, but they can usually be found in a good series of sections; in sections of parasitized larvæ I have been entirely unable to locate them.

Otherwise the parasite appears to have no effect on the internal organs of the host, with the exception of displacing the spinning glands, the largest portions of which normally lie in the position later occupied by the parasite. Although they may be so displaced as to lie dorsad of the alimentary tract (Plate IV., Fig. 9, *b*) their functioning is in no way impaired, since these larvæ spin quite as many silken threads as do those which are uninfested.

The *Mermis* probably feeds directly on the blood plasma and fatty tissue of the host.

As the larva reaches maturity the contained worm becomes very restless, and if a living larva be placed under the low power of a microscope in a cell slide the movements of the worm can be easily observed. One thus watched for about half an hour was seen to explore the hypodermis of the host, evidently attempting

to find a place of exit. The head was kept constantly moving over the internal surface of the abdominal hypodermis and was even sometimes thrust far into the thoracic region. The movements appeared to cause the host great pain, especially when the thoracic region was visited. Finally after the entire worm had been several times twisted round in the body the head was pressed against the junction of the third and fourth abdominal segments and a hole was quickly made through which the worm slowly emerged. The worm, measuring 3 cm., took in all 27 minutes to disengage itself after puncturing the skin of its host. At first the operation was very slow and the constant writhing and turning of the host impeded rather than aided the movements of the parasite. When about 50 mm. were exposed the head was twisted around the body and the remainder of the worm was more rapidly forced out, finally becoming detached from the host in a tightly knotted mass which soon straightened out. The larva meanwhile rapidly shrank, and in about half an hour was dead. The worm apparently can leave the abdomen at almost any point, though the thin junction between two segments is usually chosen. It is probable that the death of most parasitized larvæ is directly due to the escape of the worm, which in all observed cases occurred some time before maturity.

On raising leaves in the bed of a stream a little below a large colony of *Simulium* larvæ it was found that they had under them several of these white worms. This year most of the worms had escaped by the beginning of May, and have since been lost sight of, though while examining a stone microscopically for a second brood of *Simulium* eggs, on May 29, one or two minute worms were seen, which may have been young *Mermis*. No *Simulium* eggs could be observed. At a still later date, August 3, a full grown, though dead, *Mermis* was found under a stone in the bed of the stream.

#### PERCENTAGE OF LARVÆ INFESTED.

Several leaves covered with larvæ were taken from a stream in Forest Hills for the purpose of determining the percentage of parasitism.

These larvæ, which represented the species *Simulium hirtipes*,

gave the following results: Number of larvæ present 174; number of parasitized larvæ 41. This means that in this particular locality about 23.5 per cent. of the larvæ would be unable to mature on account of the *Mermis* parasite.

The species living in the Stony Brook Reservation was found to be parasitized only to the extent of some 3-4 per cent. A few *S. hirtipes* were also present in the same stream and were parasitized only to the same extent, so it is probable that this smaller attack was not due to the host's belonging to a different species.

#### CAUSE OF THE RETARDATION OF THE HISTOBLASTS.

In order to arrive at some definite conclusion as to why the histoblasts of a parasitized larva should not develop normally, it will be of advantage to review briefly some of the theories which have been advanced to explain the normal development of these discs, and at the same time to consider the facts which have been brought to light by the study of several cases now on record in which the discs have developed with abnormal rapidity, thus producing larvæ which possess characters that normally do not make their appearance until the pupal or adult condition is reached. This abnormal condition has been termed "prothetely" by Kolbe (1903).<sup>1</sup> For the abnormally retarded condition as produced in the *Simulium* larvæ by the presence of *Mermis* I should like to suggest the word "metathetely."<sup>2</sup>

The earliest report of prothetely was made by Heymons ('96). In this case larvæ of *Tenebrio molitor* L. were found which, when mature, proved to be abnormally developed as follows:

1. The meso- and meta-thorax possessed expanded lateral portions of the tergites, which resembled the wing-pads of the pupa, though they were not folded under the body as in the latter, but were directed backwards.
2. The antennæ had additional incompletely segmented joints, thus approaching the 11-jointed adult condition.
3. The abdominal tergites were modified so that they resembled the tergites of the pupal abdomen.

These larvæ were raised in the mealworm cultures of the

<sup>1</sup> Προθεῖν, to run before, and τέλος, the completion.

<sup>2</sup> Μεταθεῖν, to run behind, and τέλος, the completion.

Berlin Zoölogical Institute, but no statement is made as to whether conditions during development were quite normal. Heymons concludes his paper by suggesting that the abnormal development described above is due entirely to an accelerated development of the histoblasts, but makes no suggestions as to the cause of the acceleration.

In 1903 Kolbe reported and figured an interesting case of prothetely in the larva of *Dendrolimus pini* L. He received the larva when in its fourth moult, at which stage it had the following characters:

1. The larval antennæ were replaced by elongate antennæ showing simple primary division into about seven segments.
2. The larval thoracic legs were replaced by three pairs of jointed legs possessing all the adult parts, namely, coxæ, trochanters, femora, tibiæ and tarsi.
3. The mouth parts were modified.

Kolbe points out that all these organs were in an immature adult, or true *pupal* form, thus showing, it would seem, that the development was quite normal, but simply accelerated. Winne-guth succeeded in breeding from a similar larva an adult which hatched as a small male that was apparently quite normal except for its dwarf size. These abnormal larvæ were produced from an artificially hatched generation kept indoors and from parents which did not hibernate.

Hagen ('72) gives an account of silkworm larvæ obtaining wings before pupation, which condition was accompanied by other abnormalities as follows: The head was small and had two small facettèd eyes, and the thorax became modified but the abdomen remained in the normal condition of a larva in the fourth moult. The fore wings were long and narrow and rather more gray than usual, while the hind wings were short and narrow. As this anomaly occurred frequently and was therefore liable to be of economic importance, its causes were investigated by Majoli who came to the conclusion that it was due to the larvæ being kept at a temperature above the normal.

A case similar to that of Heymons was described by Riley ('08) from another coleopterous larva, *Dendroides canadensis*, which was bred by a student at Cornell University.

It will be seen from these cases that in every instance they occurred in artificially reared larvæ, and it is probable that the prothetely was due to an increased temperature, perhaps with the aid of abundant food, in some way hurrying on the development of the histoblasts.

This fact suggests that the histoblasts may be caused to develop, though at a much slower rate than the larval organs until pupation, by some enzyme secreted by the insect and that they can develop only as fast as this stimulant is formed. Its supply thus acts as a regulator. An increased temperature is in most cases advantageous to enzyme action and in this case it is probable that it either causes more of the enzyme to be secreted or stimulates the action of the amount already available.

Dewitz ('05), after numerous experiments on retardation and acceleration of development and pupation arrived at the following conclusions: Development and pupation are caused by enzymes which are not very evident in the early larval stages. They, however, increase with the growth of the larva until its pupation, at which period they are at their maximum strength; they then begin to diminish, till at the end of the pupal period their action entirely ceases. He also states that the enzyme action can be hindered by the presence of another body; and that by obtaining an enzyme of an increased strength before pupation development can be abnormally accelerated.

It is evident that the cells of the histoblasts are caused to develop by a different stimulus from that which causes the development of the larval organs, for in the former case development is very slow during the larval stage, when the latter organs are developing very rapidly, and it is only when these have reached their limit of development at pupation, that the adult organs begin to develop with any rapidity.

This suggests that there may be in the insect body two sets of enzymes which one may term "larval" enzymes and "imaginal" enzymes. These are sufficiently different so that conditions which cause the acceleration or retardation of one of them need not necessarily have any effect on the other. If this be so, one has a probable explanation of prothetely and also, as I shall attempt to show in the following paragraphs, of metathetely....

At first sight one would suppose that the retardation in development of the histoblasts in parasitized *Simulium* larvæ is simply due to a lack of proper nourishment for these organs, since the larva, besides having to supply the requirements of its own developing larval tissues, has also to supply the demands of its fast-growing parasite. This may be true to a certain extent, but later observations, when another parasite is also present, indicate that there must be some other more potent factor which accounts for this inhibition. This second parasite is a Sporozoön which, owing to the vast numbers in which it occurs in a single host, is far more bulky than the worm and must, one would imagine, make far greater demands on the resources of its host. In this case, however, the histoblasts are usually unaffected in size, though in many cases they are distinctly smaller than normal. Two individuals, however, were seen in which the histoblasts were minute. On dissection it was found that, in each case, a small worm measuring only some 7 mm. was living embedded in the mass of spores, and it was evident that this minute worm was responsible for the retarded condition of the discs, even though it had evidently absorbed very little nourishment. One must, therefore, in all probability look to some *toxin* secreted by the worm as the cause of the inhibition of development in the discs.

The researches of Verson and Bolle, as quoted by Fischer ('06), proved in the case of lepidopterous larvæ that in their early stages their body fluid is alkaline and that this alkalinity decreases as the larva matures. This would suggest that an alkaline condition encourages the growth of larval tissues whereas acidity, or the absence of alkalinity, permits of the development of the adult organs. Hence the histoblasts would develop but slowly till the larvæ are nearing maturity when the decreased alkalinity of the blood allows the "adult" enzymes to stimulate the cells of these discs to rapid division. I have been unable to find any account of the excretions of *Mermis* or of closely related Nematelminthes, but should they be proved to have an alkaline reaction the probability of the above contention would be very greatly strengthened, for here we should have a case of the alkalinity of the system being maintained in maturing larvæ, and thus preventing the normal, though slow, development of the

adult organs, without affecting the larval organs to any great extent, except in so far as they are kept well supplied with alkaline fluids and are thus capable of developing to their utmost extent. This would account for the somewhat larger size of parasitized individuals.

It may be, however, that the *Mermis* does not actually secrete an alkaline substance, but brings about an increased alkalinity in the body of its host by absorbing whatever acids are formed in it. The probability of this being the true condition is increased by the fact that closely related worms live in acid media, such as vinegar or sour paste (*Anguillula aceti* Chrbg.), which would point to the fact that the worm requires, and absorbs, acids during its development. In either case the effect on the host would be the same in that there is a reduction in the activity of the acids which appears to be essential to the development of the histoblasts. Whether this is the true explanation or not, it is certain that the presence of the worm does have a direct inhibitory effect upon the development of the imaginal organs, but does not have a similar effect on the larval tissues.

A suppression of pupal and adult organs will naturally be of advantage to the parasite for two reasons:

1. Nourishment is not required for building up these tissues and therefore more will be available in the body cavity of the host.

2. The maturity of the host will be deferred thus giving the parasite a longer life, should it require extra time for development. In most of the observed cases, however, the worm killed its host, by emerging before or at about the same time that the uninfested larvæ were pupating.

A similar though less marked case of metathetely due to parasitism by *Mermis* has been described and figured by Mrázek ('08) in the queens of a European ant (*Lasius alienus*). In this case the parasitized larvæ matured and produced adult ants which were normal in all external characters with the exception of a great reduction in the size of the wings. Through the kindness of Professor Wheeler I have been able to examine some similarly parasitized specimens of a closely related American species (*Lasius neoniger*) in order to see whether the development of the

legs had been in any way affected but a careful comparison of the measurements of the legs of the parasitized ants with those of healthy specimens failed to reveal any inhibition of their development on account of the *Mermis*, although the wings, which in normal ants measured some 10–11 mm. in length were reduced in the parasitized individuals to 6–6.5 mm.

In the case of *Simulium* larvæ, as before stated, the development of the legs also is inhibited by the presence of *Mermis*, though comparative measurements of the wings and leg histoblasts in healthy and parasitized larvæ show that whereas in the former case the wing histoblast covers about four times the area covered by that of the mesothoracic leg, in parasitized larvæ these histoblasts bear a relation to each other in size of about 2.5 : 1, showing that the wing histoblast suffered a greater inhibition in development than did that of the leg.

A further interesting case of *Mermis* parasitizing ant larvæ was described by Wheeler ('10), in which case the worker larvæ of *Pheidole commutata* were parasitized, and resulted in the adults of such larvæ not only possessing all the normal "worker" characters perfectly developed, but owing to excess of feeding on account of their constant hunger these "worker" larvæ developed, when mature, characters such as the ocelli which are normally only found in the sexual ants.

#### A SPOROZOÖN PARASITE OF SIMULIUM LARVÆ.

While dissecting out worms from a batch of larva taken on March 30, I chanced to cut open one larva which from the whiteness of the abdomen I took to contain a worm, but was much surprised to find that the body cavity was closely packed with a white substance which had the appearance of cotton wool. A little of this substance, however, when smeared on a slide was seen to be composed of countless organisms as illustrated in Plate V., Fig. 14. My first impression, very naturally, was that these were spermatozoa, which they resemble very closely in general outline. It was, however, very difficult to imagine to what possible organism these spermatozoa could belong. The larva itself could surely not produce them; but if not the larva what then? The only explanation seemed to be that they were

formed by one of the worms, which could not then be *Mermis* but must be a new form closely related to *Allantonema* or *Sphaerularia*, in the females of which the uterus becomes protruded and is finally many times the size of the original worm.

I naturally visited the stream in order to obtain more specimens suffering from this disease, only to find that a quantity of oil which had been flowing down the stream for some days past had succeeded in killing off all the larvæ in that neighborhood. It was therefore necessary to find a place further up the stream, above the contamination, where the larvæ were living. About half a mile's walk led to such a place, situated under a stone arch, in which the larvæ were present literally in thousands. They formed great masses covering the whole surface of the rocks. An examination of a few rocks soon showed that the *Mermis* was very plentiful here, but it was some time before I found specimens with the curious white abdomens for which I was searching. I chanced, however, to pull up a piece of water weed that was floating in a swiftly running swirl and here I found quite a number of individuals, some showing immense abdominal swellings due to the parasite. On examining a few small specimens I found that the parasite was more numerous among them than among the larger individuals; a closer examination of the rocks showed that these small parasitized individuals were quite commonly scattered among the larger healthy ones. A quantity of material was taken back to the laboratory to be studied on the same lines as that adopted for the *Mermis* parasite.

#### EXTERNAL EFFECTS ON THE LARVA.

The first effect noticed in badly infested larvæ is the immensely distended abdomen. In some cases as in that illustrated in Plate V., Fig. 12, the apex of this region of the body was three to four times its normal size. Unlike the *Mermis*, this parasite is not confined to the ventral portion of the body but entirely surrounds the caudal extremity of the mesenteron, and small detached colonies were not infrequent in the thoracic region. The Malpighian tubules, however, can usually be seen on the surface of the mass and are plainly visible through the tightly stretched skin, which is quite transparent in places and appears to be on the point of bursting.

The length of the larvæ next claims attention. No larva containing this parasite was found to be exceptionally large; whereas, as before mentioned, many were extremely small, measuring some 2.75 or 3.5 mm. at a time when all normal larvæ measured some 9 mm. or more. This may possibly require a similar, though opposite, explanation to that suggested on page 281 to account for the large size of the individuals affected by the *Mermis*.

In confinement these small specimens were extremely restless, they were continually twisting about and moving over the sides and bottom of the jar with a peculiar jerky movement. The larger, and more heavily parasitized, larvæ on the other hand were more sluggish though not more so than the healthy larvæ.

The effect of these parasites on the histoblasts is very hard to state with any certainty. Fig. 13 is an illustration, made with a camera lucida, of the histoblasts of a nearly mature individual. In this case the parasite was confined to the abdomen. It will be seen that the discs are but half developed (compare with Fig. 6). In other cases, however, the development of the discs was not, so far as could be seen, affected in any way till pigmentation of the respiratory filaments commenced. Then it was noticed that the entire histoblast only turned a slate gray color instead of blackening at the apex of the filaments and finally over the complete disc. In other cases, however, the discs entirely blackened in a perfectly normal manner.

When colonies of parasites are located in the thorax, as is not infrequently the case, the histoblasts are materially decreased in size, while in the very small specimens development appeared to have been arrested. This is hardly surprising. My general conclusions were that the parasite affected the histoblasts but little, though the larvæ rarely advanced so far toward maturity that the respiratory filaments became pigmented. It should be noted that the power of spinning silk was in no way affected by the presence of this parasite.

#### NATURE OF THE PARASITE.

While visiting other localities I made numerous observations on the larvæ to be found in the streams, and was much surprised to find that species of the parasite were extremely common and

that in almost every brook visited some of these conspicuously distorted individuals were to be found. On making microscopical examinations I observed that the organisms taken from different localities varied very much in form and apparently represented different species. A list of these different forms and their hosts is appended:

1. Ovoid bodies bearing a "flagellum"-like organ varying in length from about that of the "body" to three times its length (Plate V., Fig. 14). Host: *Simulium hirtipes*. Habitat: Forest Hills and Blue Hills, Mass.

2. Bi-annulated ovoid bodies bearing a "flagellum," which is never much longer than the "body" (Plate V., Fig. 15). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

3. Ovoid bodies having the "flagellum" replaced by a transparent flattened disc (Plate V., Fig. 16). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

4. Simple ovoid bodies destitute of all appendages (Plate V., Fig. 17). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation and Blue Hills, Mass.

The following characters were common to all of these organisms:

1. They were all similar in size.

2. Under the highest power of the microscope they had a faint olive greenish tinge.

When in a fresh condition absolutely no internal details were visible and in specimens which had been killed, fixed and stained very little more could be seen. In many of the first or "spermatozoid" type a darkened central area indicated the presence of a very large nucleus, while a smaller dark spot just before the base of the flagellum might be taken for the nucleolus.

Many of the "simple ovoid" type, although but slightly staining, showed a great shrinkage of the internal substance; otherwise nothing could be seen in them. The specimens with a flattened disc, and those bearing two annuli and a flagellum, also showed practically no differentiation. Methyl green and various hæmatoxylin combinations were tried without success.

Besides these various forms, no two of which were observed

to occur in the same individual, another form of cell (Plate V., Fig. 18) was found in much smaller numbers, but possibly in some way connected with them, for it was seen in association with each form, but was not found in healthy larvæ. This cell, which varied much in diameter from but little more than the numerous "spores" to three or four times the length of their longest axis, was apparently globular in form, and in a fresh state was very transparent and could only be discerned with difficulty. The following characters, however, were seen. The substance of the cell was finely granular, and often contained a number of large transparent globules. When fixed and stained the only differentiation was that of a large dark mass, apparently the nucleus (Plate V., Figs. 18, *a* and *b*). In some cases these cells seemed to be dividing (Plate V., Fig. 18, *c*).

In the face of all these diverse types of cell, which live in precisely the same way, it is evident that the forms first found are not spermatozoa, and further the fact that the flagellum is replaced in one "species" by a flattened disc eliminates the possibility of their being *Flagellates*. It is therefore probable that one must look to the *Microsporidia* among the *Sporozoa* as the group to which these bodies belong. It is further noticed that many of the forms are very similar to those met with in the genus *Glugea* to which the well known *pébrine* disease (*G. bombycis*) of silk-worms belongs. One must therefore conclude that it is a pebrine-like disease, which is killing off a high percentage of the Simuliid larvæ in the neighborhood of Boston, though on account of the vast difference in structure and mode of life of the two hosts the life history of the parasite in the *Simulium* larvæ is very unlike that described by Pasteur ('70) and Stempell ('09) in their work on the disease in silk-worms.

#### LIFE HISTORY OF THE PARASITE IN ITS HOST.

As in the case of *Mermis*, I know very little about this, since I did not discover it till the final stages of the larva were being approached, and in every instance but one it was apparently in exactly the same condition, namely, the "spores" were all formed and were simply awaiting the death of their host and its resulting decomposition to escape into the water.

The one exception, however, was that of a larva found on April 17. It was one of the first discovered, in the abdomen of which could be seen, with a dissecting lens, large white bodies floating in the blood plasma. On dissection these bodies proved to be rounded masses of flagellate spores, a few of which had become disengaged and were apparently moving about by their own impetus in the blood plasma. As, however, I have never on any subsequent occasion observed such a movement among these spores I am inclined to believe that this was simply a Brownian movement, which I have distinctly recognized in later examinations. Though I searched carefully for other larvæ with parasites in a similar stage I was unsuccessful.

In serial sections of parasitized larvæ similar effects to those exhibited when *Mermis* is present, are noticed in that the fat-body is much reduced (Plate IV., Fig. 10) and the sexual organs could not be detected, whereas the spinning glands and musculature apparently remained unaffected. The parasite probably enters the host through the alimentary tract for in all cases of infected larvæ it was found that the mesenteron was distorted in one or more places showing distinct hypertrophy as if the cells had been badly irritated but had healed over again often permitting the spores to pass through into the body cavity (Plate IV., Fig. 10, e). In one case a small colony of "spores" was found inside the alimentary tract, but it is quite possible that these had been recently taken in with the water, as parasitized larvæ were constantly dying in the colony, and any material, floating in the water such as liberated "spores," would be caught by the cephalic fans of still living larvæ.

In all recorded cases of Microsporidia parasites it has been noticed that the "spores" live, at least during their early stages, inside some body tissue of the host. An example of this is to be found in pébrine of silk-worm larvæ, in which case the spinning glands are the main seat of attack. It must be borne in mind, however, that an attack upon these organs in the larva now under consideration would, in all probability, result in the early death of the host, for it is only by means of the much strengthened silk threads that the larva is enabled to maintain in rapid streams the perpendicular position which is essential to obtaining food,

while at the same time the larva has to depend to a large extent on these threads for retaining any foothold on the rocks, for when moving its position, the adhesive disc of the proleg frequently loses its grip and the larva is washed clear of the rock. Very rarely, however, the anchoring threads, which are always present, break so that the larva is able slowly to draw itself once more to its support. Sections of the *Simulium* larva disclose the fact that there are very few other tissues in the body. The muscular system is much reduced, and the only tissues available for attack without rapidly killing the host are the fat-body and the sexual organs, and I am under the impression that it is the latter which are usually the original seat of attack. In frontal sections of a very young larva taken during March, only one testis could be found, but on the other side of the body a small mass of minute cells, taken at the time for small oenocytes, was situated a little back of the normal position of the missing testis. The cells are too small to show any structures but it is possible that this is a diseased testis to which the spores made their way as soon as they entered the body cavity. Later sections also show traces of a very thin membrane investing the mass of "spores."

I kept a large number of diseased larvæ in running water, hoping to see some of them pupate, but in every case they died, and soon liberated the spores, whereas many of the healthy larvæ, which were approaching maturity, pupated in captivity. It is thus evident that heavily parasitized larvæ never pupate, but die in the stream, liberating their countless spores in the water. What happens to these spores I have been unable to ascertain. Larvæ were allowed to die in distilled water and the liberated organisms were examined at intervals, but though they strongly resisted decomposition they never showed signs of movement or altered condition. Sections of pupæ and adults obtained from badly infested localities failed to reveal any cases of the disease being carried beyond the larval stage. It is therefore probable that the disease is not hereditary as is the case with pébrine, and as before stated it was seen that the presence of this parasite, in every case examined, apparently resulted in castration of the host. It is, however, possible that among the vast numbers of larvæ a few were only slightly parasitized and did not have their

sexual organs entirely destroyed. Such individuals should be capable of pupation and might in that way carry the disease over as in the case of pébrine. An examination of several hundreds of larvæ did not reveal one in which such a condition was possible.

Were there overlapping generations of larvæ the maintenance of the parasite could be more easily understood, but the spring brood pupated and hatched during the first half of May, and there are not at the time of writing any signs of more eggs being deposited on the rocks. It thus seems that there must be a secondary host in which this parasite passes the summer.<sup>1</sup>

#### THE PERCENTAGE OF INFESTED LARVÆ.

The number of infested larvæ varied to a great extent in different streams. In the part of the stream in Forest Hills where the parasites were first noticed, less than 1 per cent. of the larvæ were parasitized, half a mile further up the stream a little under 40 per cent. were infested, while in the Stony Brook Reservation where two types of "spores" were present among the parasitized larvæ, between 70 and 80 per cent. of the individuals were to be seen with the immensely distended and whitened abdomens which proclaimed them to be suffering from this fatal disease.

It will thus be seen that should this disease together with the previously described *Mermis* parasite, prove to exist as abundantly in other localities as it has during the past spring in the vicinity of Boston it must be of considerable importance in the natural control of the black flies which are such an annoyance to man and beast, especially in more tropical regions, and if the supposed secondary host of the Sporozoön does not prove to be a fish or animal of any value it should be possible to infect streams where Simuliid larvæ breed, and diminish their numbers very largely without the danger of poisoning the fish by applying oil or other substances to the water.

#### SUMMARY.

*Simulium* larvæ, which are found in vast numbers in small rapid streams around Boston, are seen to feed by standing per-

<sup>1</sup> I have since July 31 noticed isolated specimens of a different species of larva in one of the streams where the parasite abounded in the spring brood, but have not found any in which there were signs of the parasite being present.

pendicularly on rocks to which they are attached by a strong anal adhesive disc, and kept in position by silken threads secreted by the salivary glands. While thus anchored they spread out a pair of cephalic fans which act as strainers and collect small particles of food from the water. The head capsule is moulted independently of the body cuticle and exposes a new capsule which is at first white with a few dark spots on the vertex, but which rapidly becomes uniformly darkened all over. The thorax bears unusually well defined and large histoblasts of the imaginal wings, halteres and legs, and also on either side a histoblast of the pupal respiratory filaments, which by turning black when the larva is mature becomes very conspicuous at this stage of growth. The larvæ are infested by two parasites, namely a *Mermis* and a Sporozoön, both of which live in the body cavity.

The *Mermis* does not affect the larval development to any extent, except by slightly increasing its size, but it inhibits the development of the histoblasts to such an extent that pupation becomes impossible.

The embryo worms are probably caught by the cephalic fans of the larvæ and pass into the alimentary tract, through the walls of which they bore and live in the body cavity of the host till the latter matures. They then rupture the abdominal cuticle and pass into the water where they live a free life under stones in the bed of the stream. The number of worms contained by a single larva is usually only one, but as many as twelve have been found. A single worm measures 3 cm., which is about three times the length of the host. In some streams 25 per cent. of the larvæ were infested with this parasite. Parasitized larvæ never pupate, but are killed by the worms when they escape.

The retardation in the development of the histoblasts is the opposite condition to that met with in prothetely which is usually caused by keeping larvæ at an abnormally high temperature. This probably results in an increased supply of the enzymes which cause these histoblasts to develop. The *Mermis* apparently excretes some substance which lessens the supply or action of these enzymes and leads to metathetely.

The Sporozoön parasite occurs in several forms in different

localities. All these forms, however, live in the same way and appear to be related to the pébrine disease of Lepidoptera. The body, especially near the apex of the abdomen, becomes much distorted and swollen on account of its interior being closely packed with a white wooly material which on dissection is seen to consist of countless "spores" of minute size. Such parasitized larvæ are usually rather smaller than healthy individuals, but the histoblasts do not appear to be much affected. The parasite apparently enters the body cavity in the same manner as that described in the case of *Mermis*. Evidence of this is seen in a hypertrophied condition of parts of the mesenteric wall. From here it seems to pass to one or both of the sexual organs which are destroyed and become the nuclei for the great mass of spores which eventually fills the abdomen. The parasitized larvæ in this case also were never observed to pupate but died when mature. The spores are liberated by a rupture of the abdominal wall soon after the death of the host and pass into the water, after which stage they have not been seen. Up to 80 per cent. of the larvæ in some streams were found to contain large masses of this parasite but no cases of slightly parasitized larvæ were observed. There has been no second brood of *Simulium* larvæ this year, so it would seem that if the parasite is to appear next year there must be a secondary host in which the summer is passed.

#### POSTSCRIPT.

The foregoing account of the Sporozoid parasite of *Simulium hirtipes* was very kindly reviewed for me by Professor G. N. Calkins, of Columbia University. From mounted specimens of the spores sent him he confirmed my opinion that these represent some species of Myxosporidea, and drew my attention to a paper by Louis Léger ('97), which I had overlooked because of its not being catalogued under *Simulium* in the cards of the "Concilium Bibliographicum" in the Cambridge library. In this paper a new species of *Glugea* parasitizing the larvae of the European *Simulium ornatum* is described as *G. varians*. The notes on this species are in brief as follows: The abdominal region of the infested larva is greatly distended and contains large masses of a free parasite in the form of opaque, milky white, irregular sacs.

Some larvæ contain but one mass whereas others contain two to four. The muscles are unaltered, but the fat body is much reduced. The alimentary canal always appears to be contorted. In only one case had the Microsporidea failed to sporulate and they then formed a swelling on the external intestinal surface. The sacs contain countless ovoid, refractive spores, which, when treated with iodine, show a filament 15-20 times as long as their longest diameter. Spores of two sizes are present, the smaller measuring 4-5 $\mu$ , the larger 8 $\mu$ . In a subsequent note written in collaboration with Hagenmueller ('08) Léger states that the spores are sometimes present in a polysporic and sometimes in an octosporic arrangement. These authors also refer to a similar parasite in the larvæ of *Tipula gigantea*.

From the foregoing notes it will be seen that the disease which occurs in *S. hirtipes* is very similar in its main features to that described by Léger, and I do not hesitate to regard the organism responsible for its occurrence as a closely related form. Professor Calkins is inclined to consider the various forms I have described as belonging to a single species. For this I would propose the name *Glugea polymorpha* sp. nov. Future investigation, however, will quite possibly show that the various forms occurring in different localities are not all representatives of the same species, since numerous dissections of diseased larvæ showed that certain types of spores were peculiar to different localities even though present in two different species of *Simulium* larvæ.

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## EXPLANATION OF PLATE I.

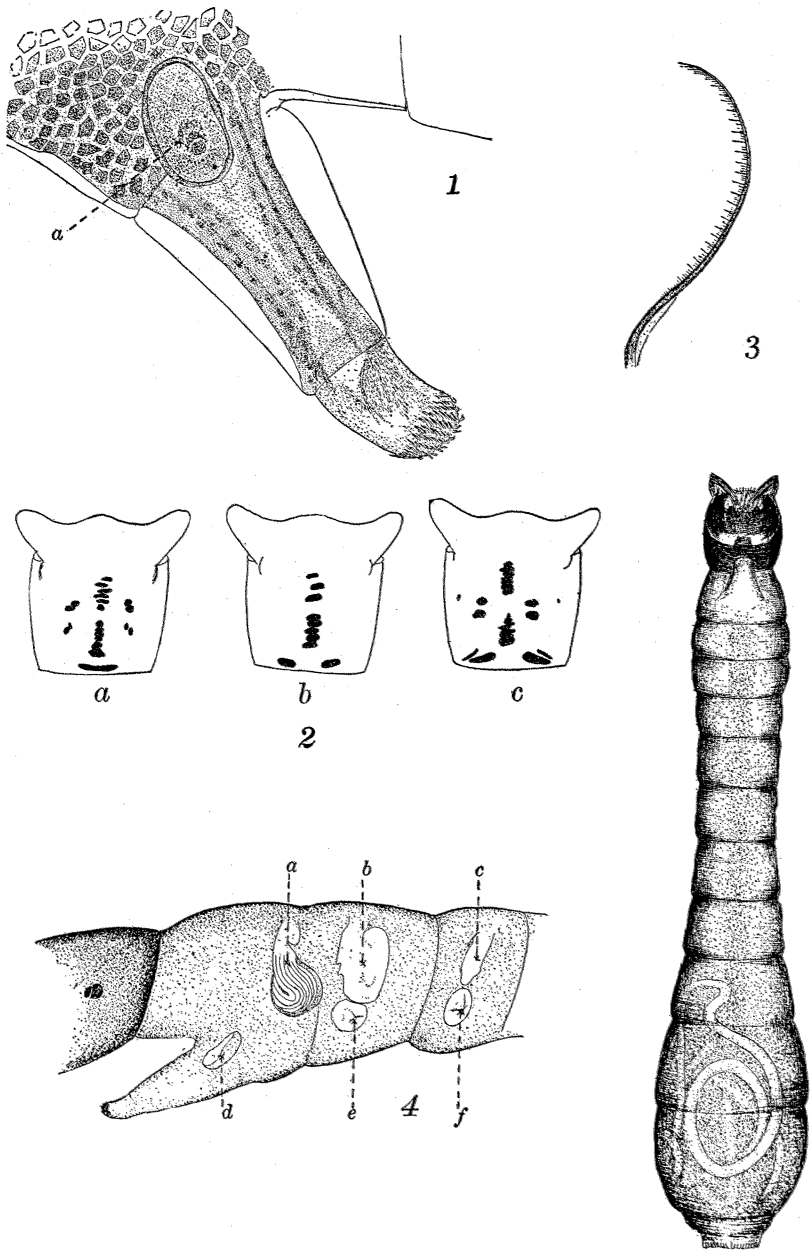
FIG. 1. Prothoracic leg of a *Simuliid* larva showing the histoblast (*a*) at an early stage of development.

FIG. 2, *a*, *b* and *c*. Types of spotting on the head of a recently moulted larva of *Simulium hirtipes*.

FIG. 3. A single rake from the cephalic fans.

FIG. 4. Profile of thorax of a half-matured larva showing the histoblasts. *a*, respiratory organ histoblast (pupal organ); *b*, wing histoblast (adult organ); *c*, halterer histoblast (adult organ); *d*, *e* and *f*, pro-, meso- and metathoracic leg histoblasts (adult organs).

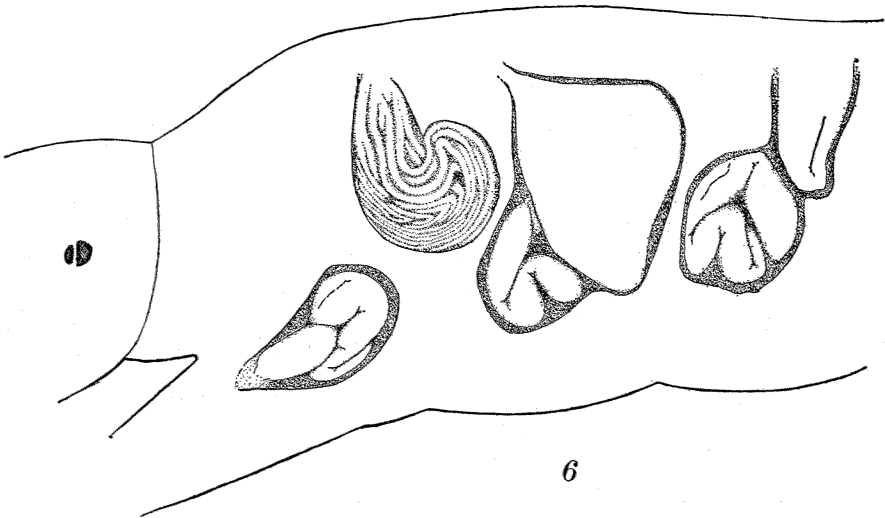
FIG. 5. Ventral view of a *Simulium* larva with *Mermis* parasites in situ.



## EXPLANATION OF PLATE II.

FIG. 6. Histoblasts of a healthy full-grown larva measuring 10.5 mm.

FIG. 7. Histoblasts of a larva parasitized by *Mermis* measuring 10.5 mm.

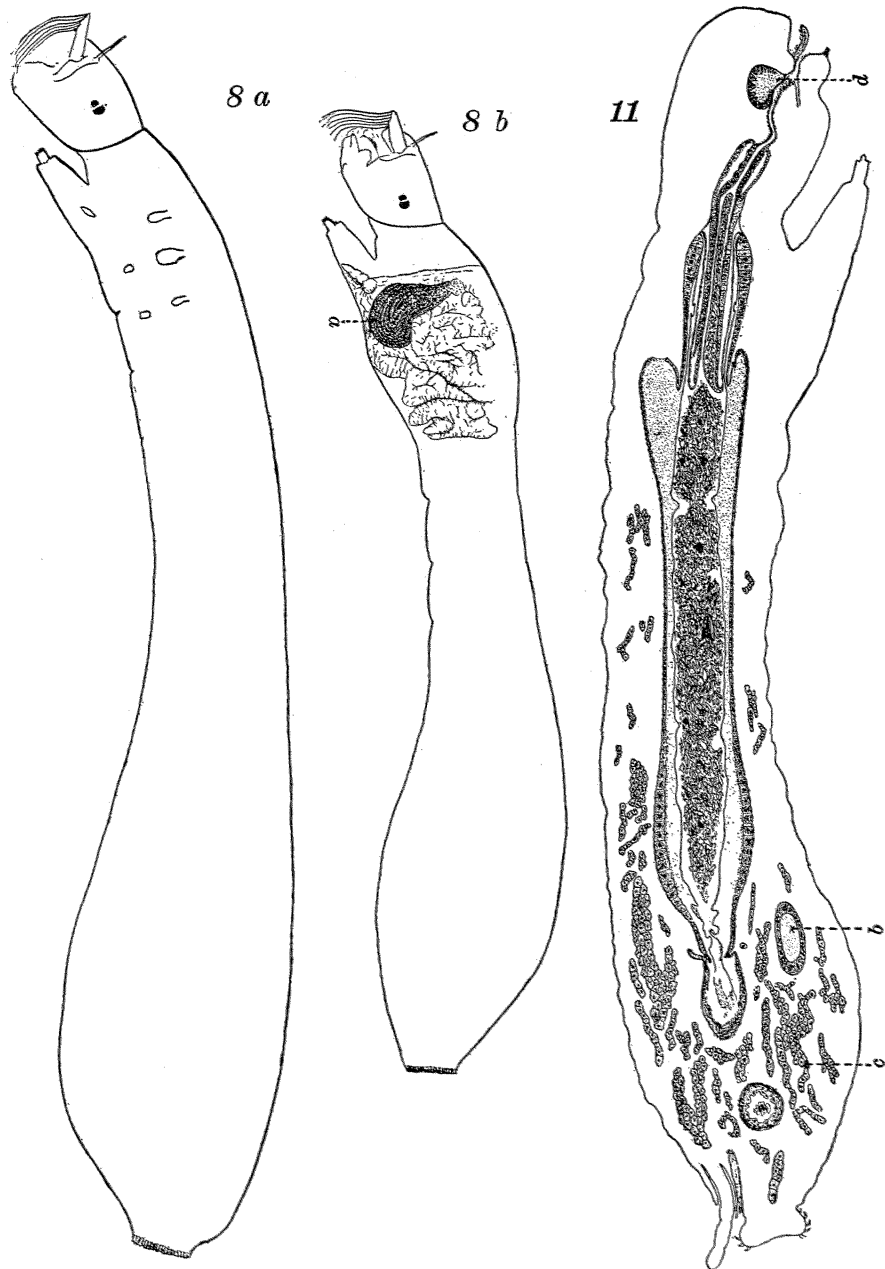


## EXPLANATION OF PLATE III.

FIG. 8*a*. Average size full-grown parasitized larva of *Simulium hirtipes* measuring 11 mm.

FIG. 8*b*. Average size mature larva of *Simulium hirtipes*, measuring 8.5 mm. showing darkened pupal histoblast, *a*.

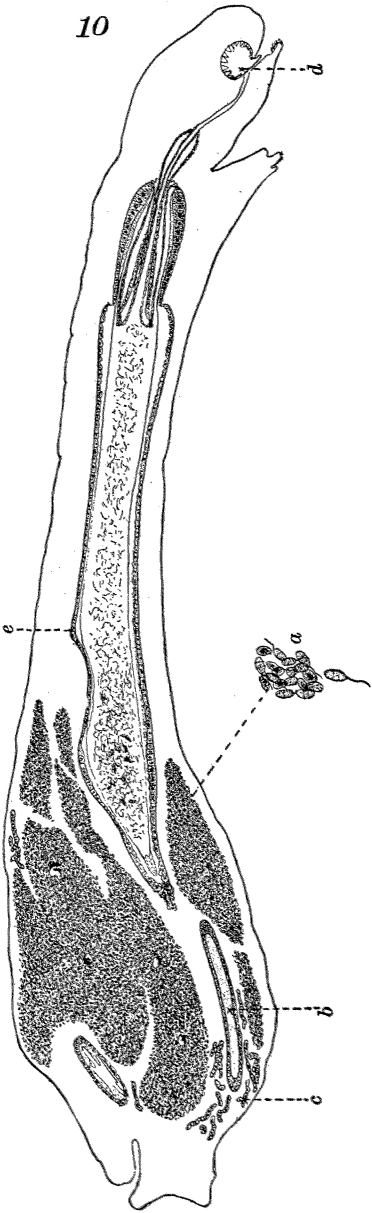
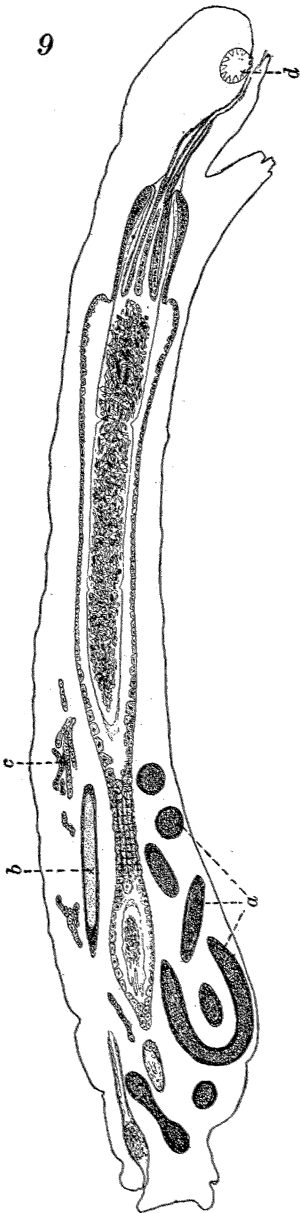
FIG. 11. Median sagittal section of a half-grown healthy larva to show the alimentary tract. *b*, the normal position of the spinning glands, which extend backwards from the pharynx on either side latero-ventrally to the alimentary canal, doubling back on themselves at about the point *b*, where one has been cut in cross section; *c*, the normal quantity of fat body which increases still more as maturity approaches; *d*, one of the pharyngeal glands. (This is not seen in an exact median section, as the two glands are narrowly separated medially.)



## EXPLANATION OF PLATE IV.

FIG. 9. Median sagittal section of a larva parasitized with *Mermis* sp. to show the somewhat displaced alimentary tract, and *a*, the coiled up worm; *b*, the displaced spinning gland, a short longitudinal section of which has been cut; *c*, the much reduced fat-body; *d*, one of the pharyngeal glands.

FIG. 10. Median sagittal section of a larva parasitized with a sporozoön to show *a*, the mass of spores, a few of which are shown enlarged; *b*, the spinning gland; *c*, the much reduced fat body; *d*, one of the pharyngeal glands, and *e*, the somewhat distorted wall of the alimentary tract.



## EXPLANATION OF PLATE V.

FIG. 12. Dorsal view of a *Simulium* larva with *Sporozoid* parasites in situ. Compare with Plate I., Fig. 5, which is normal in shape.

FIG. 13. Histoblasts of a larva parasitized with the *Sporozoa* measuring 9 mm. Compare with Plate II., Fig. 6.

FIG. 14, A, B and C. Simple "flagellate" type of parasite,  $\times 4,000$ .

FIG. 15, A and B. Annulate "flagellate" type of parasite,  $\times 4,000$ .

FIG. 16. Type of parasite with "flagellum" replaced by a flattened disc, A showing surface view of disc,  $\times 4,000$ ; B showing side view of disc,  $\times 4,000$ .

FIG. 17, A, B, C and D. Simple ovoid type of parasite,  $\times 4,000$ .

FIG. 18, A, B and C. Other bodies found in small numbers among the "spores,"  $\times 4,000$ . Many are larger in proportion than those illustrated. C shows one apparently dividing.

